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REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY AND HEPATOLOGY

Regulation of Hepatic Stellate Cell Phenotypes in Metabolic Dysfunction–Associated Steatohepatitis

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Hepatic stellate cells (HSCs) play a crucial role in the pathogenesis of liver fibrosis in metabolic dysfunction–associated steatohepatitis (MASH), a condition characterized by excessive fat accumulation in the hepatocytes, unrelated to alcohol consumption. In a healthy liver, HSCs are quiescent, store vitamin A, and function as pericytes. However, in response to liver injury and inflammation, HSCs become activated. In MASH, HSC activation is driven by metabolic stress, lipotoxicity, and chronic inflammation. Injured hepatocytes, recruited macrophage, capillarized sinusoidal endothelial cells, and permeable intestinal epithelium may each contribute to activating HSCS. This leads to a unique inflammatory environment that promotes fibrosis. MASH HSCs change their metabolism to favor glycolysis, glutaminolysis, and lactate generation. Activated HSCs transform into myofibroblast-like cells, producing excessive extracellular matrix components that result in fibrosis. In addition, HSCs in MASH have inflammatory and intermediate activated phenotypes. This fibrotic process is a key feature of MASH, which can lead to cirrhosis and liver cancer. Understanding the mechanisms of HSC activation and their role in MASH progression is essential for developing targeted therapies to treat and prevent liver fibrosis in affected individuals.

Keywords: Liver Fibrosis; Myofibroblast.

Metabolic syndrome, obesity, and insulin resistance[1–3] contribute to the pathogenesis of metabolic dysfunction–associated steatohepatitis (MASH), causing hepatic steatosis, ballooning degeneration of hepatocytes (steatosis), lobular inflammation, fibrosis, and cirrhosis.[4] From 60 to 80 million Americans are estimated to have metabolic dysfunction–associated steatotic liver disease (MASLD), a spectrum of liver diseases ranging from steatosis to nonalcoholic steatohepatitis MASH with fibrosis and cirrhosis, to hepatocellular carcinoma.[1] It remains unclear why some patients have a higher predisposition to rapid fibrosis progression. Several mouse models of MASH have been developed to study the pathophysiology of MASH using advanced genetic and pharmacologic interventions (reviewed in Vacca et al[5]).

Pathogenesis of Liver Fibrosis

Liver fibrosis studies in patients and in experimental models have revealed common pathophysiological mechanisms that include chronic metabolic injury of hepatocytes, development of endoplasmic reticulum stress, reactive species production, excessive lipid accumulation, hepatocyte death,[2,3] increased intestinal permeability with gut dysbiosis, activation of liver resident Kupffer cells (KCs), recruitment of bone marrow–derived neutrophils and myeloid cells, and secretion of inflammatory (including interleukin [IL] 6, tumor necrosis factor [TNF], IL1β, IL17, and IL13) and fibrogenic (transforming growth factor [TGF]-β1, CTGF, platelet-derived growth factor [PDGF], and leptin) cytokines.[2] Inflammatory and fibrogenic cytokines facilitate de novo lipogenesis and steatosis in hepatocytes[4] and alter the crosstalk between hepatocytes and liver sinusoidal endothelial cells (LSECs) and hepatic stellate cells (HSCs).[6,7] These changes are associated with a decline in liver function,[2,8–10] loss of LSEC fenestration,[11] and activation of quiescent HSCs (qHSCs) into collagen type I–secreting myofibroblasts, which produce fibrous scar and make liver fibrotic.[10,12–14]

Regression of Liver Fibrosis

Clinical observation in patients and experimental models have demonstrated that liver fibrosis may regress after the removal of causative agents.[10,15–17] Regression of liver fibrosis is associated with decreased inflammatory and fibrogenic responses, increased collagenase activity,

Abbreviations used in this paper: **ACC, acetyl-CoA carboxylase; aHSC, activated hepatic stellate cell; apoE, apolipoprotein E; α-SMA, α–smooth muscle actin; CCl_4, carbon tetrachloride; CLOCK, circadian locomotor output cycles kaput; CoA, coenzyme A; CPT1A, carnitine palmitoyltransferase 1A; ECM, extracellular matrix; Fas, Fas receptor; GFAP, glial fibrillar acidic protein; HSC, hepatic stellate cell; iHSC, inactivated hepatic stellate cells; IL, interleukin; INF, inflammatory-activated subcluster; KC, Kupffer cell; LRAT, lecithin:retinol acyltransferase; LSEC, liver sinusoidal endothelial cell; MASH, metabolic dysfunction–associated steatohepatitis; MASLD, metabolic dysfunction–associated steatotic liver disease; mRNA, messenger RNA; PDGF, platelet-derived growth factor; PPARγ, peroxisome proliferator-activated receptor–γ; PROLIF, proliferating; qHSC, quiescent hepatic stellate cell; RAR, retinoic acid receptor; RXR, retinoid X receptor; sEV, small extracellular vesicle; TF, transcription factor; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; TREM, triggering receptor expressed on myeloid.**

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resorption of the fibrous scar, and disappearance of myofibroblasts through apoptosis or inactivation. Besides the disappearance of myofibroblasts, another important component of liver fibrosis regression is the change in phenotype of macrophages.[18] Extracellular matrix (ECM) degradation critically depends on the influx of bone marrow–derived myeloid cells that produce matrix metalloproteinase-2, matrix metalloproteinase-9, and other matrix metalloproteinases that cleave collagen type I fibers.[18] Prolonged liver injury produces irreducible collagen crosslinking, for example, formation of ECM, which is uncleavable for collagenases, and prevents resorption of fibrous scar and regression of liver fibrosis.[18]

Hepatic Stellate Cells

Phenotypes of Hepatic Stellate Cells in Normal and Fibrosing Liver

HSCs are resident mesenchymal cells located in the space of Disse, the perisinusoidal area between endothelial cells and hepatocytes. HSC functions have been extensively characterized using experimental models and clinical analysis of patient livers. Under normal physiological conditions, HSCs exhibit a quiescent phenotype and do not express stress fibers. Resting qHSCs closely interact with hepatocytes and endothelial cells and assist in the intercellular transport of cytokines and soluble mediators. In response to injury, HSCs activate (aHSCs) into myofibroblasts and produce ECM, mainly collagen types I and III. On cessation of fibrogenic liver injury, aHSCs can follow 3 cellular fates. aHSCs can senesce as a mechanism of limiting proliferation of damaged cells. Senescent cells are usually recognized and eliminated by immune cells. If not killed by natural killer T cells, senescent cells produce inflammatory cytokines that reportedly worsen liver fibrosis.[19] Approximately 50% of myofibroblasts undergo apoptosis, and approximately 50% of HSCs inactivate and acquire quiescent-like state (iHSCs).[15,20]

Quiescent Hepatic Stellate Cells

The qHSCs express neural markers (glial fibrillar acidic protein [GFAP], synemin, synaptophysin, NGFR1, and lecithin:retinol acyltransferase [LRAT])[21,22] and lipogenic genes (peroxisome proliferator-activated receptor–γ [PPARγ], Adipor1, Adpf, C/EBPd and others), and store vitamin A.[23] Stellate cells play a key role in intrahepatic uptake, storage, and release of retinoids and store up to 70%−95% of all vitamin A in the body.[24] Formation of lipid droplets in qHSCs is dependent on the enzymatic activity of Lrat.[25] Retinol is esterified via either acyl-coenzyme A (CoA) retinol acetyltransferase that uses free retinol or by LRAT that uses CRBPI-bound retinol.[26] Mobilization of retinyl esters occurs via retinyl ester hydrolase, to produce retinol. Bound to retinol binding protein, retinol can be secreted into the circulation[27] or transported to hepatocytes.[28] Retinol and retinoic acid are important ligands for nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs).[29] In addition to retinoids, HSC lipid droplets contain triglycerides, and lesser amounts of cholesterol, cholesteryl esters, phospholipids, and free fatty acids. Similar to adipocytes, expression of PPARγ and SREBP-1c in HSCs drives fatty acid storage and metabolism.[30,31] Maintenance of the quiescent phenotype of stellate cells is dependent on sufficient expression of retinoic acid and RAR-β and RXR-α.[26,32] Fatty acids and their derivatives, such as prostaglandins, are natural ligands for PPARs.[33–35] Transcriptional regulation of qHSCs is mediated by PPARγ,[36] and activation of HSCs is associated with down-regulation of PPARγ. Expression of Lrat, PPARγ, NGFR1, apolipoprotein E (apoE), and GFAP are associated with a qHSC phenotype.[26,37]

qHSCs mediate multiple functions, including maintaining the hepatocyte/LSEC/HSC niche and homeostasis in the space of Disse, providing intercellular communications, secreting cytokines (eg, IL6 and ET1), chemokines (eg, CCL2 and CCL5), growth factors (eg, hepatocyte growth factor, vascular endothelial growth factor, insulin-like growth factor II, and epidermal growth factor), and neurotrophins (nerve growth factor, brain derived growth factor, NT-3, and NT-4/5), regulating vascular tone and sinusoidal blood flow, and promoting hepatocyte proliferation.[38] qHSCs synthesize polypeptide mediators, erythropoietin, and components of the plasminogen activation system that maintain homeostasis in the microenvironment of the hepatic sinusoid.[26,39] HSCs are required for hepatic regeneration by secreting HGF, galectins, neurotropin-3, and Respo3.[40]

Although early studies proposed that qHSCs were a homogeneous population, single-cell RNA-sequencing analysis of healthy mouse livers have identified 2 clusters of aHSCs. These studies demonstrated that gene expression in the qHSCs changes along the hepatic acinus, such that there are markers that distinguish the central vein–associated HSCs (including Adamtsl2 and RSPO3) from the portal vein–associated HSCs (including Ngfr and Itgb3).[41] The genes of HSC cluster located around the portal vein had a strong interaction at the level of basement membrane–type ECM proteins, including laminins and type IV collagen. The central vein–associated HSCs genes had strong interactions of the AP1 proteins (ie, Jun, JunB, and Fos) characteristic of HSCs,[41] but did not include ECM pathway genes. A similar result was demonstrated for normal human livers with the periportal cluster defined by expression of GPC3 and the pericentral cluster defined by expression of DBH.[42]

Activated Hepatic Stellate Cells

In response to chronic liver injury with secretion of TGF-β, qHSCs down-regulate expression of vitamin A and retinol- and lipid-associated enzymes (such as Lrat, Adipor, Adpf, and others), neural markers (eg, GFAP, synemin, synaptophysin, and nestin), and quiescence-associated nuclear receptors (RXR, farnesoid X receptor [FXR], liver X receptor [LXR], pregnane X receptor [PXR], and PPARγ) and activate into aHSCs/myofibroblasts with up-regulated expression of α–smooth muscle actin (α-SMA), Col1a1, Col1a2, TGFβRI, tissue inhibitor of metalloproteinase (TIMP) 1, Spp1, VIM, LoxL1, Spp1, PDGF receptor β (PDGFRβ), and other fibrogenic proteins; secrete ECM proteins, mainly collagens I and III; resulting in liver scarring. Moreover, aHSCs migrate from

the space of Disse to the fibrotic septa.[43] TGF-β promotes transcription of collagen type I via SMAD2/3-dependent signaling pathway.[43,44] In addition, fibrillar collagen translation and secretion is enhanced by the RNA binding protein LARP6 through the 5′ untranslated stem loop structure of the messenger RNAs (mRNAs) for Col1A1, Col1A2, and Col3A1.[45–47] Activation of HSCs into myofibroblasts also depends on JunB/AP-1, TEAD, and MEF2 transcription factors.[39] Gut permeability and Toll-like receptor 4 signaling can indirectly activate HSCs by decreasing expression of the TGF-β decoy receptor BAMBI.[48] The aHSCs also secrete TGF-β, IL6, IL1β, CCL2, and ICAM-1. CTGF, IL13, and IL17 enhance Col1a1 transcription in a TGF-β–independent manner.[49,50] IL17 also directly induces collagen type I via activation of the Stat3 signaling pathway.[50] Proliferation of aHSCs is regulated by PDGFRβ, and CCL2 plays a critical role in aHSC motility and migration.[39]

Senescent Hepatic Stellate Cells

aHSCs can acquire a senescent phenotype, a terminal cell fate in which proliferating cells undergo irreversible cell cycle arrest, but remain metabolically active. Senescence of HSCs can be induced by DNA damage, unfolded protein response, or other stress-mediated signals, or telomere shortening.[20,51,52] Senescent HSCs down-regulate collagen production, but up-regulate senescence-associated secretory phenotype[53] and secrete highly pro-inflammatory cytokines (including IL1β and IL1β-dependent IL33[54]) that further facilitate liver injury. HSCs undergo senescence after prolonged culture,[55] in experimental model of toxic carbon-tetrachloride (CCl_4) induced liver fibrosis,[56] and during regression of liver fibrosis.[57] Senescent HSCs are believed to arise from aHSCs.[54] In experimental models of liver fibrosis, senescence of HSCs is regulated via activation of p53-dependent and p16-dependent mechanisms. Mice deficient in Tp53 and Cdkn2a exhibit a defect in senescence of aHSCs and are more susceptible to liver fibrosis.[20] Insulin-like growth factor I receptor is strongly expressed in HSCs, and insulin-like growth factor I induces cellular senescence in HSCs in vitro and in vivo.

Apoptotic Hepatic Stellate Cells

aHSCs/myofibroblasts are susceptible to apoptosis and can be cleared by death receptor–mediated cell death in response to deprivation of fibrogenic stimuli. aHSCs increase expression of Fas receptor (Fas) or TNF receptor 1 and their ligands, and pro-apoptotic proteins, decreased expression of pro-survival proteins, and activate a caspase8/caspase3-signaling cascade, which results in their ultimate apoptosis.[58] Alternatively, overexpression of pro-apoptotic proteins, such as p53, Bax, and Bcl-2 leads to caspase-9–mediated programmed cell death.[10] Natural killer cells and liver-specific cells $\gamma\delta$ T mediate interferon gamma–dependent killing of HSCs.[14,59,60] $CD8^+$ tissue resident memory T cells promote fibrosis regression through Fas-FasL–mediated apoptosis of aHSCs in a mouse MASH model.[61]

Inactivated Hepatic Stellate Cells

Some myofibroblasts/aHSCs escape cell death during regression of fibrosis. They up-regulate expression of survival genes HSP90AA1 and HSPA/B1 and revert to an inactivated phenotype.[62,63] The functional role of HSP90AA1 and HSPA/B1 in HSC survival and inactivation was evaluated in vivo and confirmed in vitro. Up-regulation of HSP90AA1 and HSPA/B1 protects iHSCs from TNF-mediated apoptosis. iHSCs are present at 7 days after cessation of CCl_4 injury and constituted approximately 50% of total HSCs. iHSCs persist in the recovering liver for at least 1 month after the onset of fibrosis resolution. iHSCs down-regulate expression of fibrogenic genes (including Col1a1, α-SMA, TIMP1, Lox, and LoxL), located in the space of Disse, and up-regulate expression of some quiescence-associated genes, like PPARγ, Bambi, and NGFR1, but not the others. For example, GFAP, Adipor1, Adpf, and Dbp are not re-expressed in iHSCs at levels comparable with qHSCs.[2,43,62] In addition, iHSCs uniquely up-regulate specific genes, such as Rnd1, IL7R, Cntfr, Csf2rb, Cx3cr1, Ly86, Cxcl2, Fos, Erg1, Spic, Atf3, ApoE, and Gabra3 (compared with qHSCs or aHSCs).[41,62,64] The mechanism of HSC inactivation is not well understood. Only a few transcription factors, including PPARγ and GATA6, are implicated in driving HSC inactivation.[43]

iHSCs exhibit a phenotype that is similar to, but distinct from the quiescent state.[62,63] In support, iHSCs are prompt to reactivate when challenged with TGF-β in vitro compared with qHSCs. Mice that have recovered from toxic fibrosis and rechallenged with CCl_4 6 months later, develop more fibrosis than naïve age-matched littermates treated under the same condition. This effect was attributed to rapid in vivo activation of iHSCs into myofibroblasts compared with qHSCs. Compared with qHSCs, iHSCs are more responsive to fibrogenic stimuli and contribute to recurring liver fibrosis more effectively.[62,64] This observation suggests that chronic liver injury may cause repetitive activation and inactivation of HSCs, increasing their susceptibility to fibrogenic stimuli.

Methods to Study Cellular Heterogeneity in Metabolic Dysfunction–Associated Steatohepatitis Liver

Phenotypic changes in HSCs are regulated at the epigenetic level,[65] with DNA modifications and chromatin structure changes controlling the recruitment of transcription factors to activate or repress target genes.[66–68] Epigenomic profiling of HSCs can identify these gene regulatory mechanisms, revealing key signaling pathways, gene regulatory networks, and transcription factors involved in MASLD-associated pathology, including fibrosis and inflammation, potentially identifying new antifibrotic targets.[69,70]

Single-Cell Multiomics: Joint Profiling of Multiple Molecular Modalities

A significant advancement in single-cell epigenomic technologies is single-nucleus multiomics, which enables simultaneous profiling of multiple molecular features. These methods allow for the simultaneous measurement of 1 or more

epigenomic aspects—often alongside gene expression—in the same cell.[71–73]

Used together, these methods are particularly valuable for studying complex tissues with multiple cell types and phenotypes, each with distinct regulatory landscapes, making them ideal for investigating HSC biology. They can reveal previously unknown regulatory mechanisms and cis-regulatory elements driving HSC phenotypes, illuminating how HSCs change across MASLD and other liver diseases. Moreover, when combined with spatial technologies, epigenomic methods enable the identification and characterization of HSC subtypes, states, and their spatial relationships within tissues. This multiomic approach can reveal new signaling pathways, transcription factors, and gene regulatory networks driving liver disease progression, and through integration with genome-wide association studies,[74] identify HSC-specific pathways linked to genetic risk for MASLD.

Epigenetic Regulation of Hepatic Stellate Cell Phenotypes

HSCs possess unique plasticity that enables the differentiation from quiescent to activated, and back to inactivated phenotypes.[75] These phenotypic changes occur in HSCs without alterations in the DNA sequence, but are regulated on an epigenetic level.[76] Chromatin immunoprecipitation sequencing analysis identified transcription factors (TFs) critical for mouse qHSCs, CCl_4-induced aHSCs, and iHSCs (10 days and 1 month after CCl_4 cessation). Similar mechanisms of regulation were identified for qHSCs in healthy foz/foz mice, aHSC in MASH foz/foz mice, and MASH foz/foz mice after regression of liver fibrosis. Analysis of epigenetic H3K4me2 and H3K27ac marks determined the putative enhancers and the clusters of dynamically induced regulatory elements near key expressed genes, critical for regulation of mouse HSC phenotypes.[76] Thus, enrichment for AP-1, TEAD, and nuclear factor-κB motifs was observed in mouse aHSCs[44]; while enrichment for ETS, IRF, and FOX motifs was associated with mouse qHSC/iHSC phenotypes. ETS1/2, GATA4/6, and IRF1/IRF2 were identified as putative HSC lineage-specific TFs, which maintain the quiescent-like phenotype. ETS1 was identified as a master regulator of HSC lineage-determining TFs. In vivo HSC-specific knockout of Gata6 or Ets1 (and Ets1-target genes, Nf1 or Pparg) exacerbated development of CCl_4 fibrosis in mice, indicating that these TFs prevent HSC activation. Gata6 and Pparg drive inactivation of mouse aHSCs. Mice deficient of Gata6 or Pparg in *HSCs* exhibit a defect in resolution of CCl_4 induced fibrosis.

Hepatic Stellate Cells in Metabolic Dysfunction–Associated Steatohepatitis in Mice

Characterization of Single-Cell RNA Sequencing Hepatic Stellate Cell Clusters

Comparative transcriptomic analysis using single-cell RNA sequencing demonstrated a strong correlation between gene expression in MASH human aHSCs and mouse aHSCs from western diet–fed *foz/foz* mice.[77–79] Early studies proposed that populations of qHSCs and aHSCs are homogeneous. However, single-cell RNA sequencing analysis identified 7 clusters of qHSCs/aHSCs/iHSCs in the livers of healthy foz/foz mice, foz/foz mice with MASH, and foz/foz mice after regression of MASH[41] (Figure 1*A*). A senescent cluster (expressing the classic senescent genes p16 and p21) was not identified under any condition.

Quiescent hepatic stellate cell clusters. The qHSCs are discussed above.

Activated hepatic stellate cell clusters. Instead of the expected single cluster of aHSCs, single-cell RNA-sequencing of foz/foz mice identified 4 clusters, reflecting the heterogeneity of HSC populations in MASH. Fibrogenic cluster A1 strongly up-regulated fibrillar collagens, α-SMA, Timp 1, and represent the classic activated aHSCs/myofibroblasts. Cluster proliferative (PROLIF) expressed genes associated with ECM production and proliferation (such as CDK1). Cluster A2 was characterized by weaker expression of Col1a1 but strong expression of IRF7, Alcam, Mt1/2, Sod6, Plvap, Ramp1, Igfbp3 genes, and appeared to have an intermediate level of activation (Figure 1*A*).[41]

Because a MASH liver has multiple HSC phenotypes, there is potential for interactions between the HSC phenotypes. In fact, a reanalysis of mouse MASH HSCs revealed potential ligand–receptor interactions within a cluster (ie, autocrine regulation), but also ligand–receptor interactions between clusters (ie, paracrine regulation, in which 1 cluster expresses the ligand, and another cluster expresses the receptor) (Figure 2). To determine whether the subclusters can undergo self-activation, we focused on autocrine interactions lacking corresponding paracrine counterparts. The resulting bubble plot revealed that these autocrine-like interactions predominantly target the inflammatory-activated subcluster (INF) (Figure 1*A*, INF), whereas they are entirely absent in the myofibroblast/activated (A1) and PROLIF subclusters. In contrast, multiple paracrine interactions (without corresponding autocrine-like equivalents) are directed to the A1 and PROLIF subclusters (Figure 1*B*). Several of these pathways are known to play a role in myofibroblast activation.[83,84] For example, the laminin–CD44 interaction is specifically directed to the A1 and PROLIF subclusters. Laminin subunit α-1 is crucial for TGF-β–mediated fibroblast proliferation, myofibroblast differentiation, and ECM production.[83] Although myeloid cells, particularly macrophages, have long been regarded as the primary mediators of HSC activation, this analysis suggests that HSC subclusters also engage in direct inter-subcluster communication.

Inflammatory cluster. Cluster INF exhibits a novel phenotype, with low expression of fibrogenic genes, but high levels of inflammatory genes (Pdgfrb, CD36, Ly6C, Mrc1, CLEC). This cluster resembles the inflammatory tumor–associated fibroblasts in pancreatic ductal adenocarcinoma.[85] Therefore, we defined this cluster as inflammatory HSCs. However, the role of inflammatory HSCs in the pathogenesis of MASH fibrosis is not understood. This cluster was identified first in MASH foz/foz mice, and subsequently in

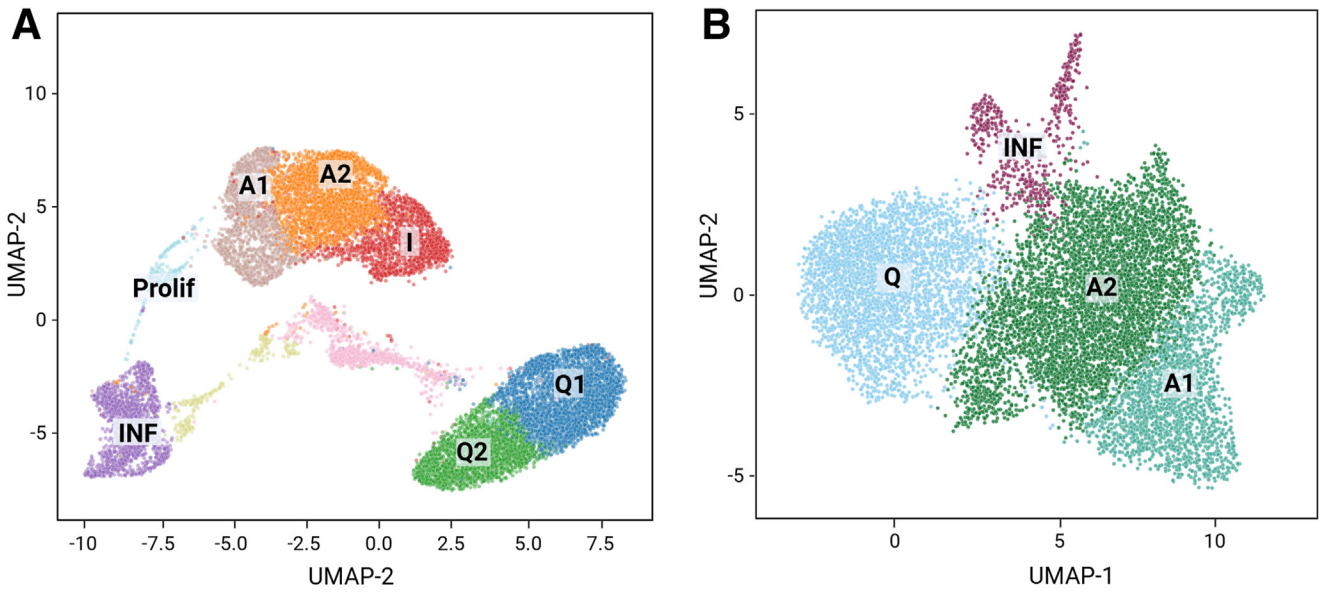

Figure 1. A simplified representation of HSC clustering in MASH by single-cell RNA sequencing. Cells plotted in uniform manifold approximation and projection (UMAP) space, cluster identity positioned at the median UMAP-1, UMAP-2 for all cells in the cluster. (*A*) HSC clustering in mouse Foz/Foz model of MASH progression and regression (based on original data in Rosenthal et al[41]). (*B*) HSC clustering in human MASH (based on original data in Kim et al[80]). A1, activated/myofibroblast; A2, intermediate or partially activated; I, inactivated; INF, inflammatory; PROLIF, proliferating; Q, quiescent.

human MASH.[80] Potential interactions between the INF cluster and other HSC clusters are discussed above.

Inactivated hepatic stellate cells cluster. One cluster of iHSCs was identified only in the livers of foz/foz mice with regression of fibrosis (Figure 1*A*). iHSCs down-regulated expression of fibrogenic genes (Col1a1, α-SMA, Timp 1, Lox, and LoxL) and up-regulated expression of quiescence-associated genes. Some genes, such as Cxcl1, Gabra3, and Fbln7, are expressed exclusively in the iHSC cluster and not in qHSCs. However, there are genes that are up-regulated in qHSCs, exhibit reduced expression in aHSCs, and then reappear in iHSCs, such as Bambi.[41]

Activation of Hepatic Stellate Cells in Metabolic Dysfunction–Associated Steatohepatitis

The classic paradigm in liver fibrosis is that the initial injury is to the parenchymal cell (metabolic dysfunction of hepatocytes in MASLD), which then activates and recruits inflammatory cells, which in turn activates HSCs. However, recent studies have demonstrated that hepatocytes, macrophage, LSECs, and enterocytes directly contribute to the activation of HSCs in MASH, including the secretion of small extracellular vesicles (sEVs, also called exosomes) that deliver cargo that modify the HSC phenotype[48,86] (Figure 3).

Hepatocyte injury is an early event in the development of MASH. Injured hepatocytes release mitochondrial damage–associated danger signals. If mitochondrial damage–associated danger signals are not cleared by macrophage, they can directly activate HSCs in vitro, and mitochondrial DNA is the most potent component. In support, patients with MASH and fibrosis have elevated blood levels of mitochondrial damage–associated danger signals.[87]

There is a strong correlation between impaired iron homeostasis and human and mouse MASH with fibrosis.[88] Human and mouse MASH hepatocytes are iron deficient, due to enhanced secretion of iron-containing sEVs. RAB27, which is a key component of sEV secretion, is highly expressed in MASH hepatocytes and knockout of RAB27 in mouse MASH restores liver iron homeostasis and decreases MASH progression. The mechanism is that uptake of these hepatocyte sEVs by HSCs results in HSC iron overload, increased reactive oxygen species accumulation and HSC activation.[89]

KCs are the predominant tissue macrophage in the normal liver. KCs are the major liver source of miR-690, and endogenous KC miR-690 is packaged into sEVs and delivered into hepatocytes and HSCs. KC miR-690 levels are markedly decreased in mouse MASH compared with KCs in normal livers, resulting in decreased miR-690 levels in MASH hepatocytes and HSCs. Decreased miR-690 levels were also found in human MASH livers compared with healthy control livers. On treatment of MASH mice with miR-690, levels of this miRNA rose in all 3 cell types. Importantly, the predominant mRNA target of miR-690 is NADK, resulting in decreased NADK mRNA and protein and a corresponding increase in NAD^+ levels. Increased NAD^+ levels inhibit activation of HSCs and liver fibrosis. Thus, normal KCs, but not NASH KCs, inhibit HSC activation.[90] Consistent with these results, incubation with niacin decreased collagen in cultured HSCs derived from patients with MASH and blocked collagen production induced by TGFβ or H_2O_2 in HSCs from normal human livers.[91]

Activation and recruitment of macrophages are early responses to hepatocyte injury that play an important role in MASH and fibrosis development. Triggering receptor

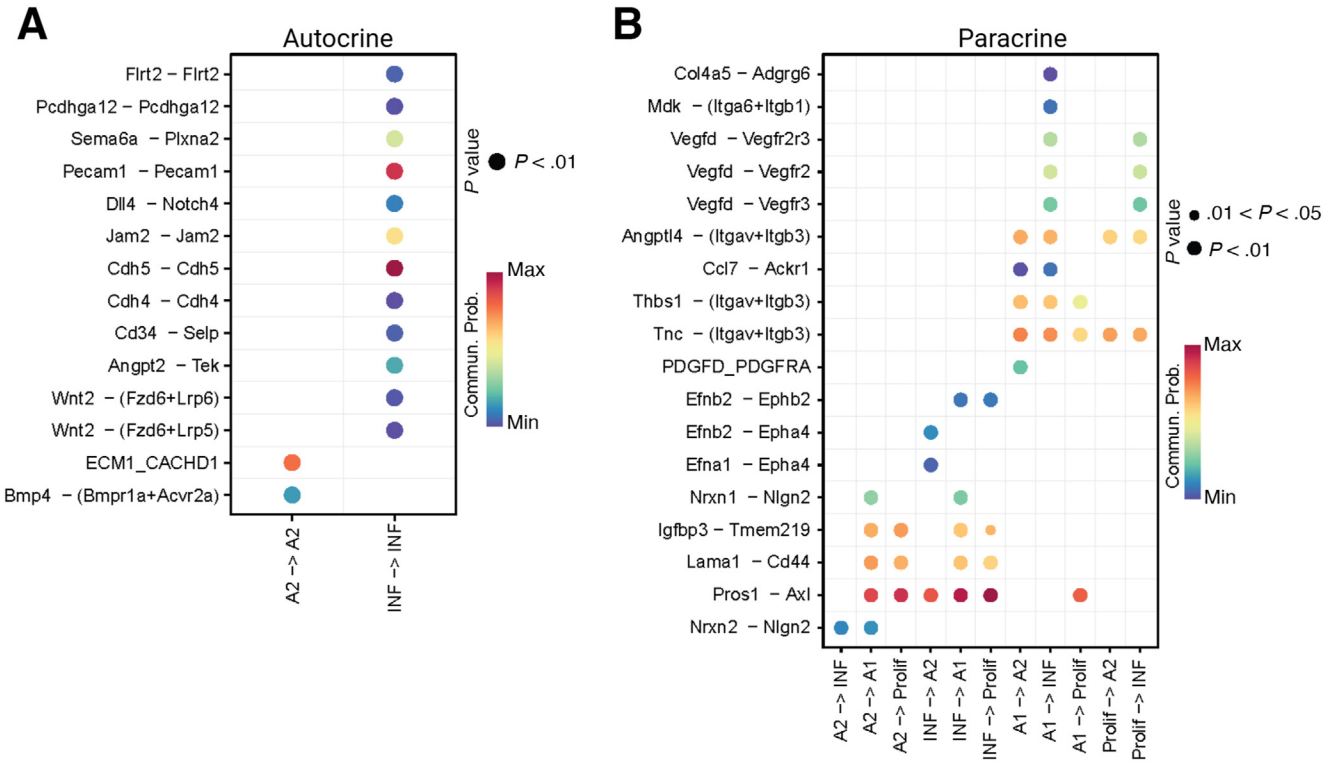

Figure 2. Predicted interactome of HSC phenotypes in MASH. *Bubble plots* show significant autocrine-like (*A*) and paracrine-like (*B*) ligand–receptor interactions among HSC subclusters in MASH samples. *Bubble color* and *size* represent the level of communication probabilities and *P* values respectively, computed using CellChat2. The analysis used the default mouse database, augmented with liver-specific ligand–receptor interactions from Xiong et al[81] and the FANTOM5 database.[82] See Figure 1 for cluster identification.

expressed on myeloid cells (TREM) are cell surface receptors that participate in diverse cellular processes.[92,93] MASH is associated with the emergence of lipid-associated macrophages that highly expresses Trem2 along with Cd9, Gpnmb, and Spp1[94–96] and are equivalent to the scar-associated macrophages in human MASH.[81,97–99] Although the Trem2 molecule itself dampens fibrosis, Trem2^{+} macrophages secrete fibrogenic factors, including TGFβ1 that activate HSCs in co-culture studies.[81] In addition, macrophages secrete prostaglandin E2 that stimulate HSC activation by binding to the EP4 receptor in a mouse MASH model.[100]

Normal LSECs suppress HSC activation and stimulate the reversion of aHSCs back toward a quiescent phenotype in vitro. The VEGF–NO and the KLF2–NO–guanylate cyclase pathways mediate this LSEC inhibition of HSC activation.[101–103] In addition, sEVs secreted by normal LSECs suppress the activated phenotype of HSCs in culture,[104] although the sEV cargo responsible for this effect is currently unknown. During MASH progression, LSECs undergo morphologic changes with the loss of their specialized phenotype and dedifferentiation. This phenomenon, called "sinusoidal capillarization," involves the loss of fenestrae (defenestration) and the formation of a basal lamina.[105] The molecular mechanisms behind capillarization leading to MASH have not been determined. Even the timing of sinusoidal capillarization during MASH progression has not been resolved. Gata4 may regulate the normal LSEC phenotype because analysis of livers from a diet-induced model of MASH showed a strong down-regulation of Gata4,[106] which was also observed in single-cell RNA sequencing from human cirrhotic livers.

VCAM1 is up-regulated in LSECs in human and mouse MASH. Deletion of VCAM1 in LSECs protects mice from diet-induced MASH in vivo and prevents HSCs activation in vitro. The soluble cleaved form of VCAM1 directly promotes HSC activation via inducing the Hippo pathway target protein Yap1. Yap1 then functions as a transcription factor to induce fibrogenic genes, such as CTGF and Ankrd1.[101] Injured LSECs secrete other factors that activate HSCs, including IL1, A-FABP, IL6, and VEGF.[107]

In experimental MASH, LSECs undergo necroptosis mediated by the downstream effector protein MLKL. Inhibition of LSEC necroptosis inhibits MASH fibrosis, mediated by decreased secretion of angiocrines, including TGFβ.[108]

Intracellular Signaling In Hepatic Stellate Cell Activation

The TGFβ/Smad pathway is the most potent pathway to activate HSCs and induce fibrosis. Other well-established morphogen pathways, hedgehog/smoothened[109] and Wnt/bcatenin,[110] contribute to HSC activation.

The more recently described Hippo pathway is critical for HSC activation in MASH. The signaling pathway is tightly controlled by phosphorylation of YAP/TAZ, which prevents its nuclear localization and interaction with TEAD1 (Figure 4). The YAP/TAZ/TEAD1 complex increases the transcription of

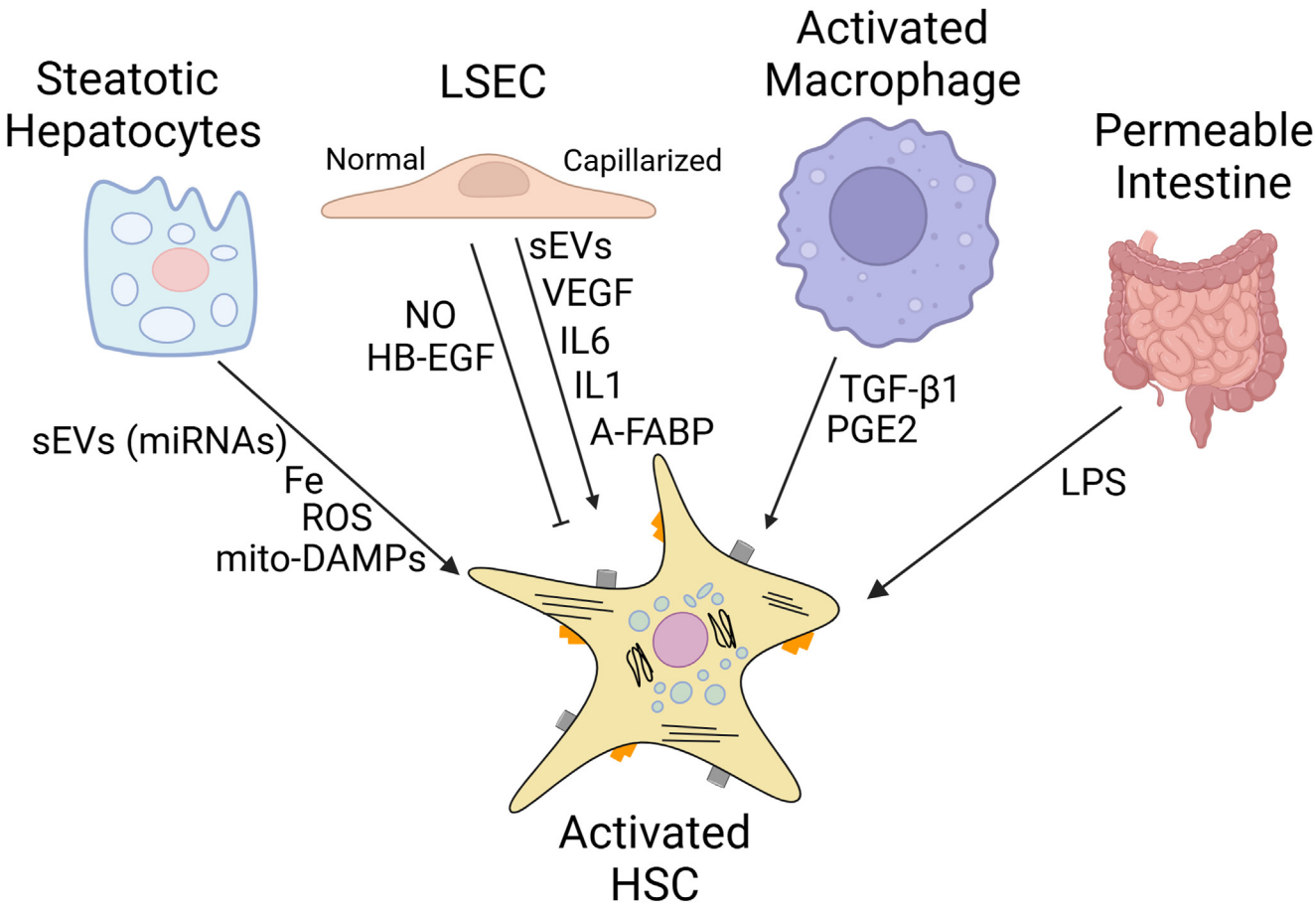

Figure 3. Paracrine stimulation of HSC activation. Multiple cell types contribute to the activation of HSCs through different pathways. A-AFABP, adipocyte fatty acid binding protein; Fe, iron; HB-EGF, heparin-binding epidermal growth factor-like growth factor; LPS, lipopolysaccharide; mito-DAMPS, mitochondrial damage associated danger signal; ROS, reactive oxygen species.

the fibrogenic genes CTGF, Cyr 67, Acta2, and CD47.[111] The Hippo pathway is also regulated at the level of mRNA splicing. The ubiquitously expressed 7-transmembrane superfamily member 3 protein (TM7SF3) forms complexes with several RNA-binding proteins/splicing factors, such as HNRNPU, HNRNPK, and RBM14. These complexes inhibit alternative splicing under basal and stress conditions.[112] TM7SF3 prevents alternative splicing of the Hippo pathway transcription factor TEAD1 by inhibiting the splicing factor hnRNPU. This results in the exclusion of the inhibitory exon 5 of TEAD1, generating a more active form of TEAD1, leading to HSC activation. TM7SF3 levels are greatly reduced in HSCs activated in MASH livers compared with qHSCs, leading to increased expression of the activated TEAD1. Preventing this TEAD1 splicing with an TEAD1 antisense oligonucleotide inhibited fibrosis in a mouse model of MASH.[112]

Hepatic Stellate Cells Metabolism in Metabolic Dysfunction–Associated Steatohepatitis

The metabolic syndrome promotes metabolic dysfunction in all tissues and cells, including HSCs. HSC activation relies on the stimulation of glycolysis, glutaminolysis, and de novo lipogenesis.

The aHSCs in MASH switch from oxidative phosphorylation to glycolysis resulting in the conversion of pyruvate to lactate (Figure 5). Glycolysis and lactate production drive HSC activation, including in mouse models of MASH. The pyruvate kinase PKM2 dimer is up-regulated in aHSCs as a result of hedgehog and hypoxia-induced factor 1α signaling. Knockdown of PKM2 markedly decreases HSC activation. The PKM2 dimer translocates to the nucleus and promote histone H3K9 acetylation, resulting in increased expression of glycolytic genes.[113] KCs have a similar switch to glycolysis during liver fibrosis.[114]

Under physiological conditions, cellular lactate levels are tightly controlled by monocarboxylate transporter 1. Monocarboxylate transporter 1 functions in HSCs to promote collagen 1 expression, as monocarboxylate transporter 1 depletion in HSCs, either in cell culture or in mice, attenuates collagen 1 protein production.[115] Hexokinase 2 expression is induced in aHSCs. The generated lactate results in histone lactylation to produce H3K18la but not histone acetylation (H3K18ac). This histone modification

results in selective increased gene expression. Inhibiting histone lactylation by hexokinase 2 deletion or pharmacologic inhibition of lactate production diminishes HSC activation, whereas exogenous lactate but not acetate supplementation rescues the activation phenotype. Thus, lactate produced by aHSCs may control gene expression via histone lactylation.[116]

Suv39h1 is a trimethyltransferase that methylates histone H3 lysine 9. Deletion of Suv39h1 in HSCs decreases liver fibrosis in MASH mice. Furthermore, co-culturing with hepatocytes exposed to palmitic acid promotes glycolysis in wild-type HSCs, but not in Suv39h1-deficient HSCs. Mechanistically, Suv39h1 facilitated the recruitment of hypoxia-induced factor 1α to stimulate the transcription of hexokinase 2 in HSCs thereby enhancing glycolysis. Consistent with these experimental results, a positive correlation between Suv39h1, hexokinase 2, and markers of HSC activation was identified in human MASH livers.[117]

GLUT1 facilitates the transport of glucose across the plasma membrane into cells. GLUT1 protein expression is increased in both mouse and human fibrotic liver tissues. In particular, immunofluorescence staining demonstrated colocalization of GLUT1 and α-SMA, demonstrating GLUT1 expression in aHSCs. TGFβ1 increased aerobic glycolysis in HSCs and induces GLUT1 expression in HSCs by activating the Smad, p38 MAPK, and P13K/AKT signaling pathways. GLUT1 inhibition blocked the effect of TGFβ1 on HSC proliferation and migration. Administration of a GLUT1 inhibitor reduced the degree of liver inflammation and liver fibrosis in a mouse model.[118]

In addition to glucose, glutamine can serve as a substrate for the tricarboxylic acid cycle. Glutaminase, the rate-limiting enzyme in glutaminolysis, converts glutamine to glutamate, which in turn is converted to α-ketoglutarate (Figure 5). Expression of genes that regulate glutaminolysis increase during activation of HSCs, and inhibition of glutaminolysis inhibits HSC activation. Blocking glutaminolysis in aHSCs suppressed mitochondrial respiration, cell growth, migration, and fibrogenesis. Supplementing aHSCs with metabolites of glutaminolysis restores these activities. Knockout of the hedgehog signaling molecule smoothened or knockdown of YAP inhibits the expression of glutaminase. Furthermore, hedgehog and YAP inhibitors block glutaminolysis and suppress HSC activation. Glutaminolysis was increased in aHSCs from patient livers and from mice with liver fibrosis. Furthermore, in mice with liver fibrosis, inhibition of glutaminase blocked accumulation of aHSCs and fibrosis progression.[119,120]

The rate-limiting step of de novo lipogenesis is the conversion of acetyl CoA to malonyl-CoA, which is catalyzed by acetyl-CoA carboxylase (ACC). Pharmacologic inhibition of ACC reduced the activation of TGFβ-stimulated HSCs in culture by preventing a metabolic switch necessary for induction of glycolysis and mitochondrial oxidative phosphorylation. Although the requirement of de novo lipogenesis for HSC activation is clear, the molecular mechanism by which inhibition of de novo lipogenesis blocks glycolysis and oxidative phosphorylation is unknown. Consistent with a direct antifibrotic mechanism in HSCs, ACC inhibition reduced liver fibrosis in a rat choline-deficient, high-fat diet model and in response to chronic diethylnitrosamine-induced liver injury (in the absence of hepatic lipid accumulation).[121]

Carnitine palmitoyltransferase 1A (CPT1A) is a mitochondrial enzyme that constitutes the rate-limiting step in the β oxidation of medium- and long-chain fatty acids to generate ATP. CPT1A expression is elevated in HSCs of patients with MASH, showing a positive correlation with fibrosis score. Similarly, CPT1A is increased in HSCs in a mouse MASH model. Furthermore, in vitro activated primary human HSCs have increased CPT1A expression. Both pharmacologic and genetic inhibition of CPT1A prevent TGFβ1- and fetal bovine serum–induced HSC activation by reducing mitochondrial activity. The overexpression of CPT1A, induced by saturated fatty acids and reactive oxygen species, triggers mitochondrial activity and expression of fibrogenic markers. Furthermore, mice lacking CPT1A specifically in HSCs are protected against fibrosis induced by a choline-deficient high-fat diet, and methionine- and choline-deficient diet.[122]

Disruption of the circadian rhythms by a western lifestyle may lead to MASLD.[123] Behavioral disruption to the circadian rhythm, for example, shift work, contributes to liver disease risk.[124] Circadian locomotor output cycles kaput (CLOCK) gene variants and related haplotypes are associated with increased susceptibility and severity in MASH.[124] Similarly, in mouse models, CLOCK is closely associated with MASH. Mice with a CLOCK mutation (CLOCKΔ19) have dysregulated circadian rhythm and develop hepatic steatosis, obesity, and the metabolic syndrome.[124] In response to CCl_4-induced liver fibrosis, CLOCKΔ19 mice displayed increased liver injury, steatosis, and fibrosis.

Similar to hepatocytes, cultured HSCs have a circadian rhythm. Cultured HSCs from CLOCKΔ19 mice have disrupted periodicity and earlier activation. qHSCs in CLOCKΔ19 appear to have a primed activated state that results in increased fibrosis after a fibrogenic stimulus. Thus, CLOCK acts to maintain a quiescent state, consistent with HSC activation correlating with circadian rhythm disruption.[124]

Phenotypes of Human Hepatic Stellate Cells in Metabolic Dysfunction–Associated Steatohepatitis

Subclustering of hepatic stellate cells in human metabolic dysfunction–associated steatohepatitis. Initial reports using single-cell or single-nucleus transcriptomics in normal to MASH human livers identified 2 HSC phenotypes, quiescent (or resting) and activated (or myofibroblastic).[97,125–128] An additional report[129] described 2 subpopulations of aHSCs, 1 less activated and 1 more activated. Using multiomics on a larger number of patients with MASH enabled characterization of the HSC gene expression profiles and epigenetic landscapes.[80] Single nucleus RNA sequencing and single nucleus assay for transposase-accessible chromatin using sequencing overlapped significantly, reinforcing the key role of epigenetic

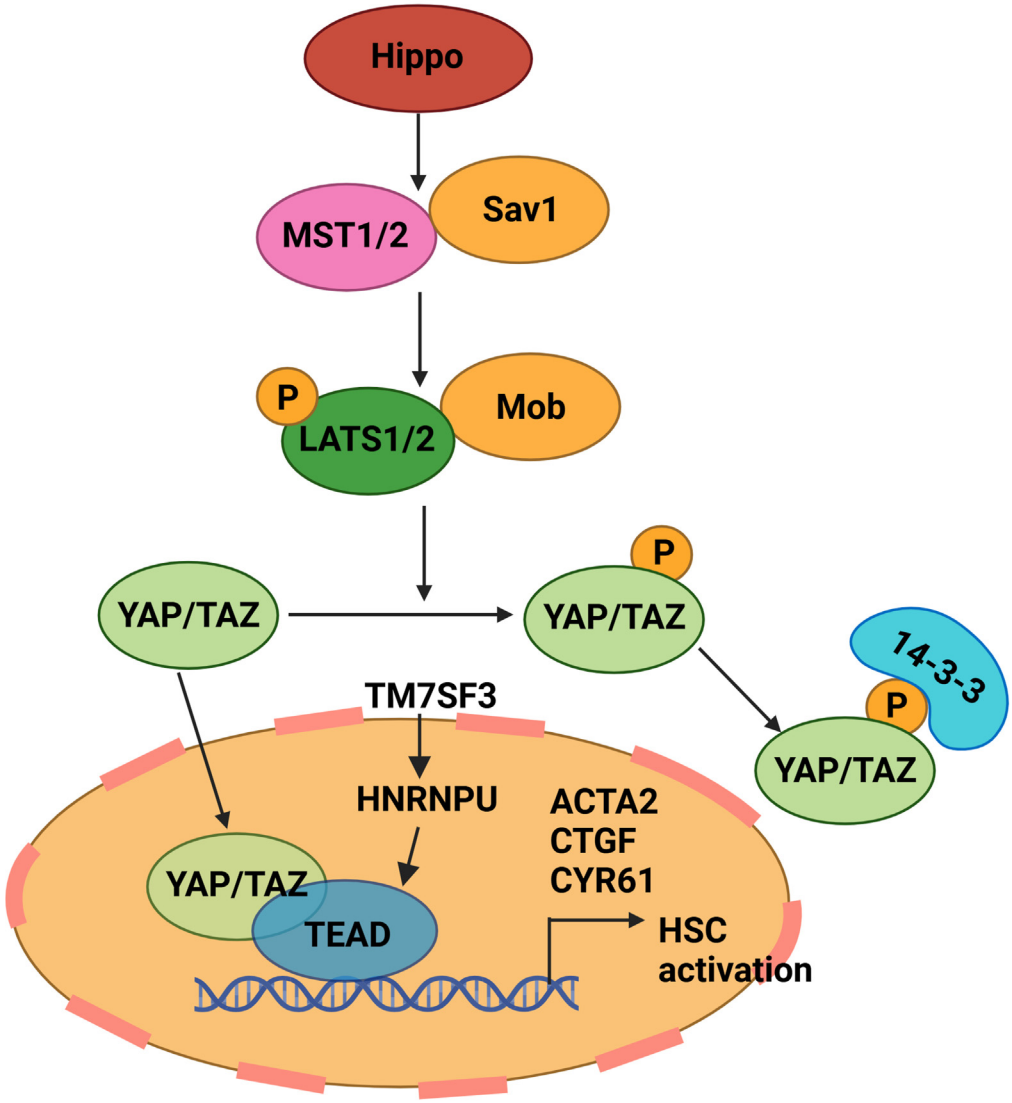

Figure 4. The YAP/TAZ pathway in HSC activation. A simplified diagram of the pathway in which Hippo activation results in YAP/TAZ translocation to the HSC nucleus. The more active form of the transcription factor TEAD is generated by down-regulation of TM7SF3.

changes in driving HSC phenotypes. Clustering revealed 1 quiescent, 2 activated, and 1 inflammatory HSC cluster in normal, MASL, and MASH livers.

Quiescent human hepatic stellate cells. Normal and MASL HSCs consist predominantly of qHSCs (cluster Q in Figure 1*B*). The quiescent cluster is distinguished from other clusters by expression of classical quiescent markers, other quiescent-associated genes (ie, LRAT, SYNM, NGFR, RELN, LRAT, RBP1, and LHX2)[80] and higher expression of the neurogenesis/vitamin A metabolic pathways. MASH livers contained a small number of qHSCs, but are highly enriched in $COL1A2^{+}LOX^{+}SPP1^{+}TIMP1^{+}$ aHSCs.[80]

Activated human hepatic stellate cells. Two activated clusters have been identified in MASH HSCs. Intermediate-activated (A2) and fibrogenic ac subclusters of HSCs (Figure 1*B*) both express fibrogenic genes, many of which play a role in ECM organization (COL1A2, LAMA4, VCAN, COL8A1, TIMP1, SERPINE1, SPON1, LAMC3, and others).[80] In general, the A2 subcluster has intermediate levels of expression of many fibrogenic genes and pathways (higher than quiescent, but lower than fibrogenic subclusters). In addition, some genes and pathways related to circulatory system development are equally up-regulated in both intermediate-activated and fibrogenic clusters of HSCs. A population of fibrogenic HSCs cluster of MASH-activated aHSCs/myofibroblasts drive ECM production. These aHSCs highly up-regulate a core group of fibrogenic genes, such as SERPINE1, SPON1, EFEMP1, GAS7, KLF9, and LTBP2.

Inflammatory human hepatic stellate cells. The INF cluster represents a small fraction of HSCs expressing CD36, CLEC, CREM, HLA-A, HLA-E, NFATC2, VAV3, SYTL3, YES1, MYO1F, FTL, FOSL2, and BTG1.[80] These genes are related to regulation of immune responses, cytokine production, actin cytoskeletal rearrangements, transcriptional alterations, and cell surface receptor signaling. The INF cluster appears to be consistently present in all human livers. The identification of an "inflammatory" HSC subset warrants further exploration to reveal functional significance and regulatory mechanisms underlying this subset in MASLD.

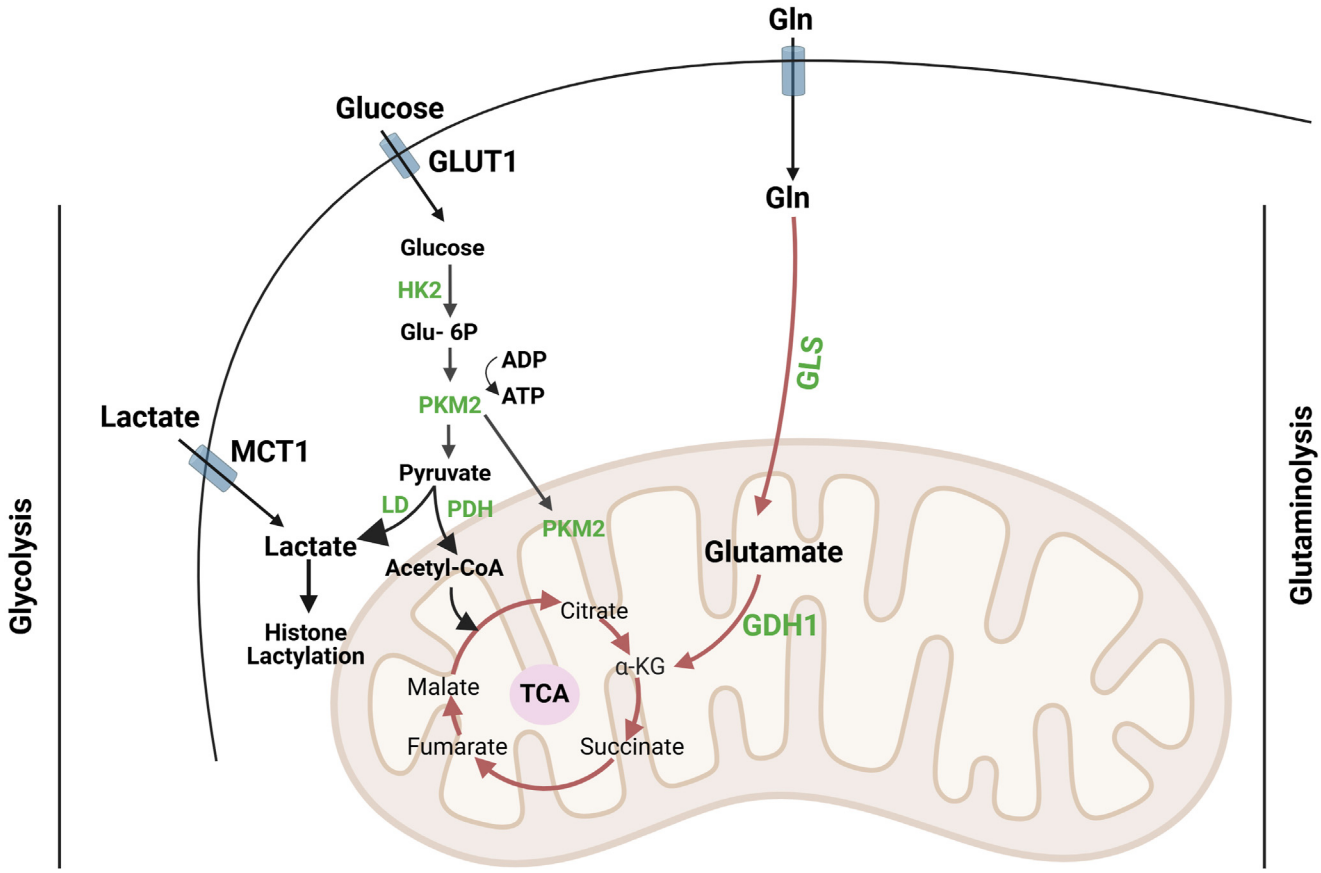

Figure 5. Metabolic changes during HSC activation. Glycolysis and glutaminolysis drive HSC activation, including by generating lactate. GDH1, glutamate dehydrogenase; Gln, glutamine, GLS, glutaminase; Glu-6P, glucose 6 phosphate; GLUT1, glucose transporter type 1; HK2, hexokinase 2; LD, lactate dehydrogenase; MCT1, monocarboxylate transporter 1; PDH, pyruvate dehydrogenase; TCA, tricarboxylic acid cycle.

Potential human senescent hepatic stellate cells. A senescent HSC cluster comprising 7% of total HSCs was reported in human MASH, but not in healthy livers.[57] These senescent HSCs up-regulate some markers of senescent cells, including GLB1, TP53, and SPI1[130–132] and uniquely express CD206.[57] However, these senescent HSCs did not up-regulate the 2 most critical markers of senescent cells $p16^{INK4a}$(CDKN2A) and p21 (CDKN1A).[20,51,133] This contrasts with senescent hepatocytes and liver sinusoidal endothelial cells in human MASH, which express high levels of p16 and p21. A very small portion of INF HSCs up-regulated some senescent genes (CD36, BCL2L1, MAPK14, CDKN1A, IGFBP2, CXCL2, BCL2L11, TP53, and AKT1),[20] indicating that some senescent-like HSCs may be present in this cluster. The same population also expressed TNFRSF10B, ASM3, PTPRC, CANX, RPS19, ZC3HAV1, TNFRSF1AB, SUSD6, CNNB1, and TNFAIP2 genes, which were implicated in facilitation of senescent phenotype in other cell types, suggesting that a percentage of INF HSCs may be primed to undergo senescence in MASH livers.

Potential human inactivated hepatic stellate cells. iHSCs were originally described in mice undergoing fibrosis regression.[62,63] Unlike mice that develop liver fibrosis within several months, progression of liver fibrosis in humans takes years and can be associated with disease regression and relapse, leading to HSC inactivation.[2] Although no definitive population of iHSCs in normal, MASL, or MASH livers were detected, a small portion of MASH HSCs in the intermediate cluster (which is less activated than fibrogenic HSCs) expressed apoE, CFH, TNXB, DHRS3, and other genes[62] that are believed to be expressed in iHSCs/qHSCs, and up-regulated some inactivation-associated genes (APOE, TNXB, DCN, ABCC9, SMOC2, GEM, RSPO3, and CYR6[41,62,63]) previously identified in mouse iHSCs. Future studies using human livers that have recovered from MASH, such as after gastric bypass surgery, are needed to characterize human iHSCs in MASH.

Regulation of Hepatic Stellate Cells Phenotypes

A conceptually novel paradigm of HSC regulation on multiple levels is the crosstalk between HSC lineage-determining, cluster-specific, and signal-specific TFs (Figure 6). A similar mechanism was developed for myeloid cells.[134] We propose that activation of fibrogenic HSCs (cluster A1) is driven by small epigenetic/transcriptional changes in the activity of lineage-determining TFs (such as increased induction of JUNB/AP-1 over ETS) that are tightly controlled by cluster-specific TFs (such as increased induction of activation-specific RUNX1/2 over quiescent-

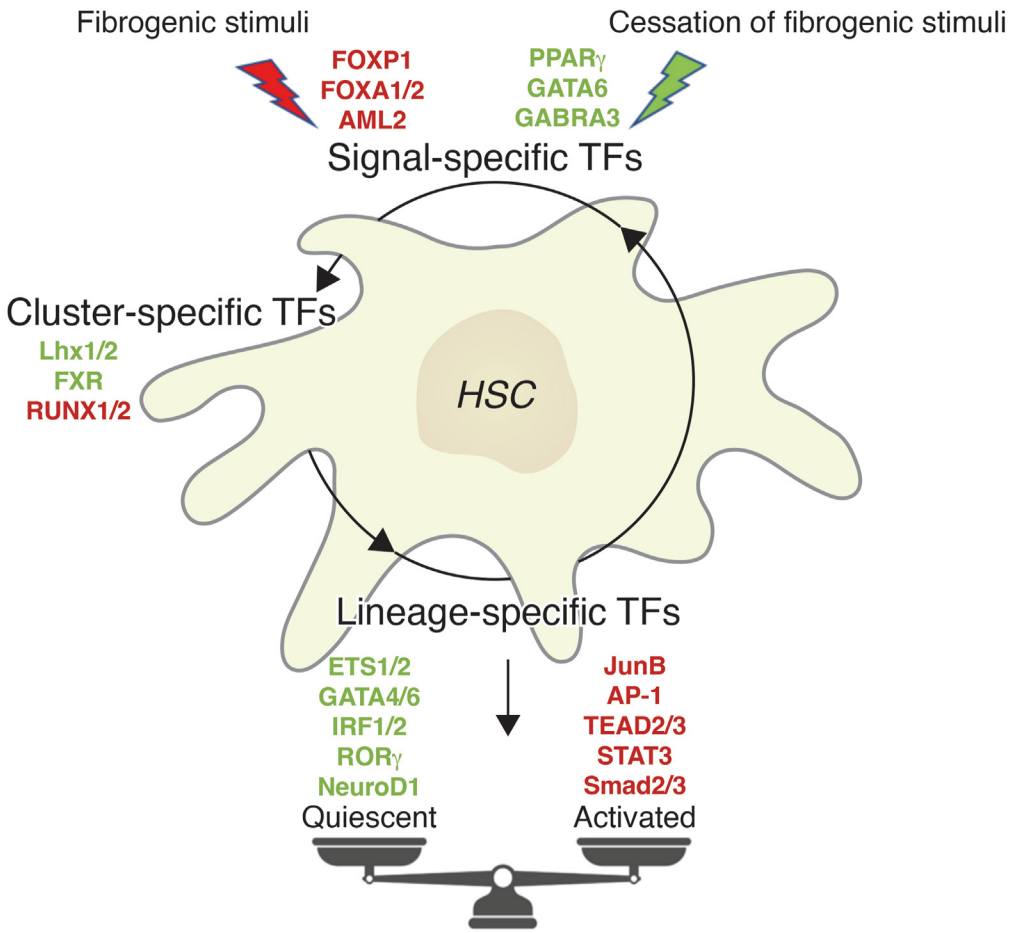

Figure 6. Proposed regulation of HSC activation via crosstalk between transcription factors. Differentially accessible lineage-determining TFs modulate HSC phenotypes. Lineage-determining TFs are abundant in specific cell types (independent of activation state), bind enhancers in a cell-specific manner, and their deletion may lead to the development of alternative cellular fate. Lineage-determining TFs *JUNB, AP-1, RUNX1, TEAD, SMAD, STAT, MEF* were previously described as associated with fibrogenic HSC phenotype, while others *ETS, IRF, GATA* were implicated in the maintenance of qHSC phenotype. Cluster-specific TFs regulate lineage-determining TFs. Cluster-specific TFs determine the phenotype of each HSC subset. *RUNX1/2* was most highly enriched in cis-regulatory elements associated with fibrogenic aHSCs, *Lhx1/2* and *FXR* was associated with quiescent aHSCs. Activation-associated *JUNB/AP1* was regulated via prevalence of *RUNX1/2* TF signaling over *Lhx1/2*, while *ETS1* was linked to increased Lhx1/2 signaling over *RUNX1/2*. In turn, cluster-specific TFs (*Lhx1/2* and others) are controlled by signal-specific TFs, which are activated in response to stimuli-driven (such as TGFβ or cessation of fibrogenic injury).

linked Lhx1/2 and FXR[76,135]), and locally regulated by signal-specific TFs (eg, TGFβ signaling that activates ECM production). Overall, different combinations of cluster-specific and lineage-determining TFs can produce a variety of signals that orchestrate HSC activation or inactivation, and mediate heterogenous HSC phenotypes in response to disease.

Identification of ATAC-sequencing peaks/candidate cis-regulatory elements that are specifically enriched in each HSC cluster followed by TF motif enrichment analysis provides a new approach to study regulation of HSC phenotypes and identified TF motifs, which are highly induced in all HSC clusters. Due to their high and ubiquitous motif enrichment in all HSCs, these TFs fall into a category of HSC-specific lineage-determining TFs (ie, JUNB, AP-1, RUNX1, RORγ, TEAD, SMAD, STAT, MEF, ETS, IRF, and GATA).[135,136] Some of these TFs were previously implicated in regulation of activated (ie, JUNB, STAT, and TEAD) or quiescent phenotypes (ie, ETS and GATA). Cluster-specific TFs are enriched specifically in each cluster (ie, Lhx1/2, FXR, RUNX1/2, and EBF). Signal-specific TFs (ie, FOX, RUNX, SREBP1, ELF3, FOXA1, and HOXA13) can co-regulate genes involved in regulation of fibrogenic responses in aHSCs, including up-regulation of SERPINE1, EFEMP1, and SPON1. Some of these TFs are directly implicated in regulation of TGFβ1-mediated fibrogenic responses.[63,135,137–139] RUNX1/2 is induced among lineage-determining, cluster-specific, and signal-dependent groups of TFs,[137] suggesting that it may be an essential master regulator of HSC activation.[140]

Comparison of mouse and human HSCs has enabled some generalizations as to the markers that identify the different cluster phenotypes, consisting of quiescent, activated, inflammatory, senescent, apoptotic, and inactivated. Single-cell analysis has further subdivided the activated phenotype into fully activated/myofibroblast and intermediate activated clusters. In the mouse MASH models, the HSC

quiescent phenotype correlates closely with normal livers, the HSC-activated phenotypes correlate closely with MASH livers, and the HSC-inactivated phenotype is exclusively found in regressed MASH livers. However, human MASH livers consist of a mixture of HSC phenotypes, such that normal livers contain aHSCs and MASH livers still contain qHSCs. Although ligand–receptor pairings may identify potential functional roles of the different HSC phenotypes within a liver, additional analysis and experimental interventions are required for mechanistic insights.

Therapeutic Approaches to Liver Fibrosis

Most antifibrotic therapeutic approaches target the secreted proteins of the aHSCs/myofibroblasts or the HSCs directly. The conventional approach is to inhibit HSC proteins associated with activation, such as increased Loxl2, type I collagen, and chaperone p47, or to induce HSC proteins that decrease on activation, such as PPARγ. More recent studies have demonstrated the antifibrotic effect of interfering with aHSC metabolism, including inhibition of hexokinase, SGLT2, acetyl Co-A carboxylase, fatty acid synthase, and stearoyl-Co-A desaturase.[141]

An alternative therapeutic approach is to induce cell death in HSC populations, such as total HSCs, aHSCs, or senescent HSCs. Deletion of the entire HSC population in mice has been tested in several studies,[142,143] resulting in improvement of liver fibrosis. However, total HSC ablation will affect liver function, disrupt liver architecture, and suppress liver regeneration.[142,144] Targeting the fibrogenic aHSCs, the major source of collagen types I and III in MASH, is a potential strategy to halt liver fibrosis while maintaining normal liver architecture. As specific pathogenic, but nonessential, HSC phenotypes are identified, their ablation can be targeted using cluster-specific ligands. Targeted ablation of senescent HSCs using urokinase plasminogen activator receptor chimeric antigen receptor cells ameliorated liver fibrosis in mice.[145] Unlike previous reports in mice,[20] our study of patients with MASH did not identify a population of HSCs expressing classic senescent markers,[80] suggesting that therapy directed at senescent HSCs requires further study.

Targeting HSCs with immunotoxins or chimeric antigen receptor T cells provides challenges due to the lack of unique markers that distinguish HSCs from other cell types in the liver. Identification of specific receptors that are expressed only on aHSCs, such as FAP,[146] could provide a new methodology for antifibrotic therapy. Alternatively, HSC specificity may be achieved by requiring 2 receptors for drug engagement.

References

1. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
2. Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. Nat Rev Gastroenterol Hepatol 2021;18:151–166.
3. Friedman SL, Neuschwander-Tetri BA, Rinella M, et al. Mechanisms of NAFLD development and therapeutic strategies. Nat Med 2018;24:908–922.
4. Kim JY, Garcia-Carbonell R, Yamachika S, et al. ER stress drives lipogenesis and steatohepatitis via caspase-2 activation of S1P. Cell 2018;175:133–145.e15.
5. Vacca M, Kamzolas I, Harder LM, et al. An unbiased ranking of murine dietary models based on their proximity to human metabolic dysfunction-associated steatotic liver disease (MASLD). Nat Metab 2024;6:1178–1196.
6. Wang X, Cai B, Yang X, et al. Cholesterol stabilizes TAZ in hepatocytes to promote experimental non-alcoholic steatohepatitis. Cell Metab 2020;31:969–986.e7.
7. Ma HY, Yamamoto G, Xu J, et al. IL-17 signaling in steatotic hepatocytes and macrophages promotes hepatocellular carcinoma in alcohol-related liver disease. J Hepatol 2020;72:946–959.
8. Brunt EM, Gouw AS, Hubscher SG, et al. Pathology of the liver sinusoids. Histopathology 2014;64:907–920.
9. Lee YA, Friedman SL. Stellate cells and fibrosis. Liver 2020:444–454.
10. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209–218.
11. DeLeve LD, Maretti-Mira AC. Liver sinusoidal endothelial cell: an update. Semin Liver Dis 2017;37:377–387.
12. Eyden B. The myofibroblast: phenotypic characterization as a prerequisite to understanding its functions in translational medicine. J Cell Mol Med 2008;12:22–37.
13. Parola M, Marra F, Pinzani M. Myofibroblast - like cells and liver fibrogenesis: emerging concepts in a rapidly moving scenario. Mol Aspects Med 2008;29:58–66.
14. Kisseleva T, Brenner DA. Mechanisms of fibrogenesis. Exp Biol Med (Maywood) 2008;233:109–122.
15. Iredale JP, Benyon RC, Pickering J, et al. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J Clin Invest 1998;102:538–549.
16. Issa R, Williams E, Trim N, et al. Apoptosis of hepatic stellate cells: involvement in resolution of biliary fibrosis and regulation by soluble growth factors. Gut 2001;48:548–557.
17. Ganguly S, Muench GA, Shang L, et al. Nonalcoholic steatohepatitis and HCC in a hyperphagic mouse accelerated by western diet. Cell Mol Gastroenterol Hepatol 2021;12:891–920.
18. Ganguly S, Rosenthal SB, Ishizuka K, et al. Lipid-associated macrophages' promotion of fibrosis resolution during MASH regression requires TREM2. Proc Natl Acad Sci U S A 2024;121:e2405746121.
19. Radaeva S, Sun R, Jaruga B, et al. Natural killer cells ameliorate liver fibrosis by killing activated stellate cells in NKG2D-dependent and tumor necrosis factor-related apoptosis-inducing ligand-dependent manners. Gastroenterology 2006;130:435–452.

20. Krizhanovsky V, Yon M, Dickins RA, et al. Senescence of activated stellate cells limits liver fibrosis. Cell 2008; 134:657–667.
21. Kendall TJ, Hennedige S, Aucott RL, et al. p75 Neurotrophin receptor signaling regulates hepatic myofibroblast proliferation and apoptosis in recovery from rodent liver fibrosis. Hepatology 2009;49:901–910.
22. Sachs BD, Baillie GS, McCall JR, et al. p75 neurotrophin receptor regulates tissue fibrosis through inhibition of plasminogen activation via a PDE4/cAMP/PKA pathway. J Cell Biol 2007;177:1119–1132.
23. Friedman SL, Roll FJ, Boyles J, et al. Hepatic lipocytes: the principal collagen-producing cells of normal rat liver. Proc Natl Acad Sci U S A 1985;82:8681–8685.
24. Hendriks HF, Verhoofstad WA, Brouwer A, et al. Perisinusoidal fat-storing cells are the main vitamin A storage sites in rat liver. Exp Cell Res 1985;160:138–149.
25. Batten ML, Imanishi Y, Maeda T, et al. Lecithin-retinol acyltransferase is essential for accumulation of all-trans-retinyl esters in the eye and in the liver. J Biol Chem 2004;279:10422–10432.
26. Geerts A. History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. Semin Liver Dis 2001;21:311–335.
27. Andersen KB, Nilsson A, Blomhoff HK, et al. Direct mobilization of retinol from hepatic perisinusoidal stellate cells to plasma. J Biol Chem 1992;267:1340–1344.
28. Ghyselinck NB, Bavik C, Sapin V, et al. Cellular retinol-binding protein I is essential for vitamin A homeostasis. EMBO J 1999;18:4903–4914.
29. Mark M, Ghyselinck NB, Wendling O, et al. A genetic dissection of the retinoid signalling pathway in the mouse. Proc Nutr Soc 1999;58:609–613.
30. She H, Xiong S, Hazra S, et al. Adipogenic transcriptional regulation of hepatic stellate cells. J Biol Chem 2005; 280:4959–4967.
31. Tsukamoto H. Adipogenic phenotype of hepatic stellate cells. Alcohol Clin Exp Res 2005;29:132S–133S.
32. Ohata M, Lin M, Satre M, et al. Diminished retinoic acid signaling in hepatic stellate cells in cholestatic liver fibrosis. Am J Physiol 1997;272:G589–G596.
33. Chinetti G, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflamm Res 2000;49:497–505.
34. Faveeuw C, Fougeray S, Angeli V, et al. Peroxisome proliferator-activated receptor gamma activators inhibit interleukin-12 production in murine dendritic cells. FEBS Lett 2000;486:261–266.
35. Lim H, Dey SK. PPAR delta functions as a prostacyclin receptor in blastocyst implantation. Trends Endocrinol Metab 2000;11:137–142.
36. Hazra S, Xiong S, Wang J, et al. Peroxisome proliferator-activated receptor gamma induces a phenotypic switch from activated to quiescent hepatic stellate cells. J Biol Chem 2004;279:11392–11401.
37. Ramadori G, Knittel T, Schwogler S, et al. Dexamethasone modulates alpha 2-macroglobulin and apolipoprotein E gene expression in cultured rat liver fat-storing (Ito) cells. Hepatology 1991;14:875–882.
38. Krenkel O, Hundertmark J, Ritz TP, et al. Single Cell RNA sequencing identifies subsets of hepatic stellate cells and myofibroblasts in liver fibrosis. Cells 2019;8:503.
39. Weiskirchen R, Tacke F. Cellular and molecular functions of hepatic stellate cells in inflammatory responses and liver immunology. Hepatobiliary Surg Nutr 2014;3:344–363.
40. Sugimoto A, Saito Y, Wang G, et al. Hepatic stellate cells control liver zonation, size and functions via R-spondin 3. Nature 2025;640:752–761.
41. Rosenthal SB, Liu X, Ganguly S, et al. Heterogeneity of HSCs in a Mouse Model of NASH. Hepatology 2021; 74:667–685.
42. Payen VL, Lavergne A, Alevra Sarika N, et al. Single-cell RNA sequencing of human liver reveals hepatic stellate cell heterogeneity. JHEP Rep 2021;3:100278.
43. Kim HY, Sakane S, Eguileor A, et al. The origin and fate of liver myofibroblasts. Cell Mol Gastroenterol Hepatol 2024;17:93–106.
44. Dooley S, Delvoux B, Streckert M, et al. Transforming growth factor beta signal transduction in hepatic stellate cells via Smad2/3 phosphorylation, a pathway that is abrogated during in vitro progression to myofibroblasts. TGFbeta signal transduction during transdifferentiation of hepatic stellate cells. FEBS Lett 2001;502:4–10.
45. Cai L, Fritz D, Stefanovic L, et al. Binding of LARP6 to the conserved 5' stem-loop regulates translation of mRNAs encoding type I collagen. J Mol Biol 2010; 395:309–326.
46. Stefanovic B, Manojlovic Z, Vied C, et al. Discovery and evaluation of inhibitor of LARP6 as specific antifibrotic compound. Sci Rep 2019;9:326.
47. Wang H, Stefanovic B. Role of LARP6 and nonmuscle myosin in partitioning of collagen mRNAs to the ER membrane. PLoS One 2014;9:e108870.
48. Seki E, De Minicis S, Osterreicher CH, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med 2007;13:1324–1332.
49. Liu Y, Meyer C, Muller A, et al. IL-13 induces connective tissue growth factor in rat hepatic stellate cells via TGF-beta-independent Smad signaling. J Immunol 2011; 187:2814–2823.
50. Meng F, Wang K, Aoyama T, et al. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. Gastroenterology 2012;143:765–776.e3.
51. Sanfeliu-Redondo D, Gibert-Ramos A, Gracia-Sancho J. Cell senescence in liver diseases: pathological mechanism and theranostic opportunity. Nat Rev Gastroenterol Hepatol 2024;21:477–492.
52. Schnabl B, Purbeck CA, Choi YH, et al. Replicative senescence of activated human hepatic stellate cells is accompanied by a pronounced inflammatory but less fibrogenic phenotype. Hepatology 2003;37:653–664.
53. Takahashi A, Loo TM, Okada R, et al. Downregulation of cytoplasmic DNases is implicated in cytoplasmic DNA accumulation and SASP in senescent cells. Nat Commun 2018;9:1249.
54. Yamagishi R, Kamachi F, Nakamura M, et al. Gasdermin D-mediated release of IL-33 from senescent hepatic

stellate cells promotes obesity-associated hepatocellular carcinoma. Sci Immunol 2022;7:eabl7209.
55. Schnabl B, Choi YH, Olsen JC, et al. Immortal activated human hepatic stellate cells generated by ectopic telomerase expression. Lab Invest 2002;82:323–333.
56. Zhang M, Serna-Salas S, Damba T, et al. Hepatic stellate cell senescence in liver fibrosis: characteristics, mechanisms and perspectives. Mech Ageing Dev 2021;199:111572.
57. Yashaswini CN, Qin T, Bhattacharya D, et al. Phenotypes and ontogeny of senescent hepatic stellate cells in metabolic dysfunction-associated steatohepatitis. J Hepatol 2024;81:207–217.
58. Iredale JP. Hepatic stellate cell behavior during resolution of liver injury. Semin Liver Dis 2001;21:427–436.
59. Kisseleva T, Brenner DA. Hepatic stellate cells and the reversal of fibrosis. J Gastroenterol Hepatol 2006;21-(Suppl 3):S84–S87.
60. Gao B, Radaeva S, Park O. Liver natural killer and natural killer T cells: immunobiology and emerging roles in liver diseases. J Leukoc Biol 2009;86:513–528.
61. Koda Y, Teratani T, Chu PS, et al. CD8(+) tissue-resident memory T cells promote liver fibrosis resolution by inducing apoptosis of hepatic stellate cells. Nat Commun 2021;12:4474.
62. Kisseleva T, Cong M, Paik Y, et al. Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. Proc Natl Acad Sci U S A 2012;109:9448–9453.
63. Troeger JS, Mederacke I, Gwak GY, et al. Deactivation of hepatic stellate cells during liver fibrosis resolution in mice. Gastroenterology 2012;143:1073–1083.e22.
64. Liu X, Xu J, Brenner DA, et al. Reversibility of liver fibrosis and inactivation of fibrogenic myofibroblasts. Curr Pathobiol Rep 2013;1:209–214.
65. Pepke S, Wold B, Mortazavi A. Computation for ChIP-seq and RNA-seq studies. Nat Methods 2009;6:S22–S32.
66. Park PJ. ChIP-seq: advantages and challenges of a maturing technology. Nat Rev Genet 2009;10:669–680.
67. Heinz S, Glass CK. Roles of lineage-determining transcription factors in establishing open chromatin: lessons from high-throughput studies. Curr Top Microbiol Immunol 2012;356:1–15.
68. Henikoff S. Nucleosome destabilization in the epigenetic regulation of gene expression. Nat Rev Genet 2008;9:15–26.
69. Mann J, Chu DC, Maxwell A, et al. MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis. Gastroenterology 2010;138:705–714; 714.e1–e4.
70. Tsukamoto H, Zhu NL, Asahina K, et al. Epigenetic cell fate regulation of hepatic stellate cells. Hepatol Res 2011;41:675–682.
71. Xiong H, Luo Y, Wang Q, et al. Single-cell joint detection of chromatin occupancy and transcriptome enables higher-dimensional epigenomic reconstructions. Nat Methods 2021;18:652–660.
72. Zhu C, Zhang Y, Li YE, et al. Joint profiling of histone modifications and transcriptome in single cells from mouse brain. Nat Methods 2021;18:283–292.
73. Xie Y, Zhu C, Wang Z, et al. Droplet-based single-cell joint profiling of histone modifications and transcriptomes. Nat Struct Mol Biol 2023;30:1428–1433.
74. Gaulton KJ, Preissl S, Ren B. Interpreting non-coding disease-associated human variants using single-cell epigenomics. Nat Rev Genet 2023;24:516–534.
75. Trivedi P, Wang S, Friedman SL. The power of plasticity-metabolic regulation of hepatic stellate cells. Cell Metab 2021;33:242–257.
76. Liu X, Xu J, Rosenthal S, et al. Identification of lineage-specific transcription factors that prevent activation of hepatic stellate cells and promote fibrosis resolution. Gastroenterology 2020;158:1728–1744.e14.
77. Arsov T, Silva DG, O'Bryan MK, et al. Fat aussie–a new Alstrom syndrome mouse showing a critical role for ALMS1 in obesity, diabetes, and spermatogenesis. Mol Endocrinol 2006;20:1610–1622.
78. Van Rooyen DM, Larter CZ, Haigh WG, et al. Hepatic free cholesterol accumulates in obese, diabetic mice and causes nonalcoholic steatohepatitis. Gastroenterology 2011;141:1393–1403.e5.
79. Mridha AR, Wree A, Robertson AAB, et al. NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice. J Hepatol 2017;66:1037–1046.
80. Kim HY, Rosenthal SB, Liu X, et al. Multi-modal analysis of human hepatic stellate cells identifies novel therapeutic targets for metabolic dysfunction-associated steatotic liver disease. J Hepatol 2025;82:882–897.
81. Xiong X, Kuang H, Ansari S, et al. Landscape of intercellular crosstalk in healthy and NASH liver revealed by single-cell secretome gene analysis. Mol Cell 2019;75:644–660.e5.
82. Ramilowski JA, Goldberg T, Harshbarger J, et al. A draft network of ligand-receptor-mediated multicellular signalling in human. Nat Commun 2015;6:7866.
83. Lee CM, Cho SJ, Cho WK, et al. Laminin alpha1 is a genetic modifier of TGF-beta1-stimulated pulmonary fibrosis. JCI Insight 2018;3:e99574.
84. Barcena C, Stefanovic M, Tutusaus A, et al. Gas6/Axl pathway is activated in chronic liver disease and its targeting reduces fibrosis via hepatic stellate cell inactivation. J Hepatol 2015;63:670–678.
85. Elyada E, Bolisetty M, Laise P, et al. Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. Cancer Discov 2019;9:1102–1123.
86. Wallace SJ, Tacke F, Schwabe RF, et al. Understanding the cellular interactome of non-alcoholic fatty liver disease. JHEP Rep 2022;4:100524.
87. An P, Wei LL, Zhao S, et al. Hepatocyte mitochondria-derived danger signals directly activate hepatic stellate cells and drive progression of liver fibrosis. Nat Commun 2020;11:2362.
88. Britton LJ, Subramaniam VN, Crawford DH. Iron and non-alcoholic fatty liver disease. World J Gastroenterol 2016;22:8112–8122.
89. Gao H, Jin Z, Bandyopadhyay G, et al. Aberrant iron distribution via hepatocyte-stellate cell axis drives liver lipogenesis and fibrosis. Cell Metab 2022;34:1201–1213.e5.

90. Gao H, Jin Z, Bandyopadhyay G, et al. MiR-690 treatment causes decreased fibrosis and steatosis and restores specific Kupffer cell functions in NASH. Cell Metab 2022;34:978–990.e4.
91. Ganji S, Hoa N, Kamanna J, et al. Niacin regresses collagen content in human hepatic stellate cells from liver transplant donors with fibrotic non-alcoholic steatohepatitis (NASH). Am J Transl Res 2022;14:4006–4014.
92. Deczkowska A, Weiner A, Amit I. The Physiology, pathology, and potential therapeutic applications of the TREM2 signaling pathway. Cell 2020;181:1207–1217.
93. Colonna M. The biology of TREM receptors. Nat Rev Immunol 2023;23:580–594.
94. Remmerie A, Martens L, Thone T, et al. Osteopontin expression identifies a subset of recruited macrophages distinct from kupffer cells in the fatty liver. Immunity 2020;53:641–657.e14.
95. Jaitin DA, Adlung L, Thaiss CA, et al. Lipid-associated macrophages control metabolic homeostasis in a Trem2-dependent manner. Cell 2019;178:686–698.e14.
96. Seidman JS, Troutman TD, Sakai M, et al. Niche-specific reprogramming of epigenetic landscapes drives myeloid cell diversity in nonalcoholic steatohepatitis. Immunity 2020;52:1057–1074.e7.
97. Ramachandran P, Dobie R, Wilson-Kanamori JR, et al. Resolving the fibrotic niche of human liver cirrhosis at single-cell level. Nature 2019;575:512–518.
98. Fabre T, Barron AMS, Christensen SM, et al. Identification of a broadly fibrogenic macrophage subset induced by type 3 inflammation. Sci Immunol 2023;8:eadd8945.
99. Zwicker C, Bujko A, Scott CL. Hepatic macrophage responses in inflammation, a function of plasticity, heterogeneity or both? Front Immunol 2021;12:690813.
100. Cao Y, Mai W, Li R, et al. Macrophages evoke autophagy of hepatic stellate cells to promote liver fibrosis in NAFLD mice via the PGE2/EP4 pathway. Cell Mol Life Sci 2022;79:303.
101. Guo Q, Furuta K, Islam S, et al. Liver sinusoidal endothelial cell expressed vascular cell adhesion molecule 1 promotes liver fibrosis. Front Immunol 2022;13:983255.
102. Nagy D, Maude H, Birdsey GM, et al. RISING STARS: liver sinusoidal endothelial transcription factors in metabolic homeostasis and disease. J Mol Endocrinol 2023;71:e230026.
103. Gao J, Lan T, Kostallari E, et al. Angiocrine signaling in sinusoidal homeostasis and liver diseases. J Hepatol 2024;81:543–561.
104. Wang J, Wu Z, Xia M, et al. Extracellular vesicles derived from liver sinusoidal endothelial cells inhibit the activation of hepatic stellate cells and Kupffer cells in vitro. Biochim Biophys Acta Mol Basis Dis 2024;1870:167020.
105. Hammoutene A, Rautou PE. Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease. J Hepatol 2019;70:1278–1291.
106. Winkler M, Staniczek T, Kurschner SW, et al. Endothelial GATA4 controls liver fibrosis and regeneration by preventing a pathogenic switch in angiocrine signaling. J Hepatol 2021;74:380–393.
107. He Q, He W, Dong H, et al. Role of liver sinusoidal endothelial cell in metabolic dysfunction-associated fatty liver disease. Cell Commun Signal 2024;22:346.
108. Yan M, Li H, Xu S, et al. Targeting endothelial necroptosis disrupts profibrotic endothelial-hepatic stellate cells crosstalk to alleviate liver fibrosis in nonalcoholic steatohepatitis. Int J Mol Sci 2023;24:11313.
109. Sicklick JK, Li YX, Choi SS, et al. Role for hedgehog signaling in hepatic stellate cell activation and viability. Lab Invest 2005;85:1368–1380.
110. Liu QQ, Chen J, Ma T, et al. DCDC2 inhibits hepatic stellate cell activation and ameliorates CCl(4)-induced liver fibrosis by suppressing Wnt/beta-catenin signaling. Sci Rep 2024;14:9425.
111. Li Y, Dong L, Yin X, et al. CD47, a novel YAP target gene, contributes to hepatic stellate cell activation and liver fibrosis induced by high-fat diet. Heliyon 2024;10:e31621.
112. Isaac R, Bandyopadhyay G, Rohm TV, et al. TM7SF3 controls TEAD1 splicing to prevent MASH-induced liver fibrosis. Cell Metab 2024;36:1030–1043.e7.
113. Zheng D, Jiang Y, Qu C, et al. Pyruvate kinase M2 tetramerization protects against hepatic stellate cell activation and liver fibrosis. Am J Pathol 2020;190:2267–2281.
114. Horn P, Tacke F. Metabolic reprogramming in liver fibrosis. Cell Metab 2024;36:1439–1455.
115. Min K, Yenilmez B, Kelly M, et al. Lactate transporter MCT1 in hepatic stellate cells promotes fibrotic collagen expression in nonalcoholic steatohepatitis. Elife 2024;12.
116. Rho H, Terry AR, Chronis C, et al. Hexokinase 2-mediated gene expression via histone lactylation is required for hepatic stellate cell activation and liver fibrosis. Cell Metab 2023;35:1406–1423.e8.
117. Wu X, Shen Y, Meng Y, et al. Suv39h1 contributes to activation of hepatic stellate cells in non-alcoholic fatty liver disease by enabling anaerobic glycolysis. Life Sci 2024;341:122498.
118. Zhou MY, Cheng ML, Huang T, et al. Transforming growth factor beta-1 upregulates glucose transporter 1 and glycolysis through canonical and noncanonical pathways in hepatic stellate cells. World J Gastroenterol 2021;27:6908–6926.
119. Du K, Hyun J, Premont RT, et al. Hedgehog-YAP signaling pathway regulates glutaminolysis to control activation of hepatic stellate cells. Gastroenterology 2018;154:1465–1479.e13.
120. Harvey LD, Chan SY. YAPping about glutaminolysis in hepatic fibrosis. Gastroenterology 2018;154:1231–1233.
121. Bates J, Vijayakumar A, Ghoshal S, et al. Acetyl-CoA carboxylase inhibition disrupts metabolic reprogramming during hepatic stellate cell activation. J Hepatol 2020;73:896–905.
122. Fondevila MF, Fernandez U, Heras V, et al. Inhibition of carnitine palmitoyltransferase 1A in hepatic stellate cells protects against fibrosis. J Hepatol 2022;77:15–28.
123. Mukherji A, Dachraoui M, Baumert TF. Perturbation of the circadian clock and pathogenesis of NAFLD. Metabolism 2020;111S:154337.

124. Jokl E, Llewellyn J, Simpson K, et al. Circadian disruption primes myofibroblasts for accelerated activation as a mechanism underpinning fibrotic progression in non-alcoholic fatty liver disease. Cells 2023;12:1582.
125. Ai L, Wang Q, Cheng K. Key genes in the liver fibrosis process are mined based on single-cell transcriptomics. Biochem Biophys Res Commun 2022;598:131–137.
126. Wang ZY, Keogh A, Waldt A, et al. Single-cell and bulk transcriptomics of the liver reveals potential targets of NASH with fibrosis. Sci Rep 2021;11:19396.
127. He W, Huang C, Shi X, et al. Single-cell transcriptomics of hepatic stellate cells uncover crucial pathways and key regulators involved in non-alcoholic steatohepatitis. Endocr Connect 2023;12:e220502.
128. Wang S, Li K, Pickholz E, et al. An autocrine signaling circuit in hepatic stellate cells underlies advanced fibrosis in nonalcoholic steatohepatitis. Sci Transl Med 2023;15:eadd3949.
129. Fred RG, Steen Pedersen J, Thompson JJ, et al. Single-cell transcriptome and cell type-specific molecular pathways of human non-alcoholic steatohepatitis. Sci Rep 2022;12:13484.
130. Wagner J, Damaschke N, Yang B, et al. Overexpression of the novel senescence marker beta-galactosidase (GLB1) in prostate cancer predicts reduced PSA recurrence. PLoS One 2015;10:e0124366.
131. Kim WY, Sharpless NE. The regulation of INK4/ARF in cancer and aging. Cell 2006;127:265–275.
132. Delestre L, Cui H, Esposito M, et al. Senescence is a Spi1-induced anti-proliferative mechanism in primary hematopoietic cells. Haematologica 2017;102:1850–1860.
133. Paluvai H, Di Giorgio E, Brancolini C. The histone code of senescence. Cells 2020;9:466.
134. Bennett H, Troutman TD, Sakai M, et al. Epigenetic Regulation of Kupffer Cell Function in Health and Disease. Front Immunol 2020;11:609618.
135. Mann J, Mann DA. Transcriptional regulation of hepatic stellate cells. Adv Drug Deliv Rev 2009;61:497–512.
136. Heinz S, Benner C, Spann N, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell 2010;38:576–589.
137. Zhang DX, Glass CK. Towards an understanding of cell-specific functions of signal-dependent transcription factors. J Mol Endocrinol 2013;51:T37–T50.
138. Wu Z, Ke Q, Jiang L, et al. TGF-beta1 facilitates gallbladder carcinoma metastasis by regulating FOXA1 translation efficiency through m(6)A modification. Cell Death Dis 2024;15:422.
139. Bergmann T, Liu Y, Skov J, et al. Production of human entorhinal stellate cell-like cells by forward programming shows an important role of Foxp1 in reprogramming. Front Cell Dev Biol 2022;10:976549.
140. Marcher AB, Bendixen SM, Terkelsen MK, et al. Transcriptional regulation of hepatic stellate cell activation in NASH. Sci Rep 2019;9:2324.
141. Parola M, Pinzani M. Liver fibrosis in NAFLD/NASH: from pathophysiology towards diagnostic and therapeutic strategies. Mol Aspects Med 2024;95:101231.
142. Puche JE, Lee YA, Jiao J, et al. A novel murine model to deplete hepatic stellate cells uncovers their role in amplifying liver damage in mice. Hepatology 2013;57:339–550.
143. Ebrahimkhani MR, Oakley F, Murphy LB, et al. Stimulating healthy tissue regeneration by targeting the 5-HT(2)B receptor in chronic liver disease. Nat Med 2011;17:1668–1673.
144. Shen K, Chang W, Gao X, et al. Depletion of activated hepatic stellate cell correlates with severe liver damage and abnormal liver regeneration in acetaminophen-induced liver injury. Acta Biochim Biophys Sin (Shanghai) 2011;43:307–315.
145. Amor C, Feucht J, Leibold J, et al. Senolytic CAR T cells reverse senescence-associated pathologies. Nature 2020;583:127–132.
146. Sun D, Li W, Ding D, et al. IL-17a promotes hepatocellular carcinoma by increasing FAP expression in hepatic stellate cells via activation of the STAT3 signaling pathway. Cell Death Discov 2024;10:230.

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Conflicts of interest
The authors disclose no conflicts.

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